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Editorial

Cyclin D1, another molecule of the year?

One of the aims of carcinogenesis research is to identify the precise molecular alterations responsible for neoplastic transformation. Normal cell cycle control is disrupted in malignant cells which are unable to regulate their proliferation correctly. Understanding how the cell cycle control operates therefore represents a fundamental problem in cancer biology. Work in recent years has led to the conclusion that cyclins, and cyclin-dependent kinases (CDKs) together with their inhibitors constitute a complicated network of positive and negative regulation circuits. These basic regulatory mechanisms are common to all mammalian cells and act at specific time points during the cell cycle (Figure 1).

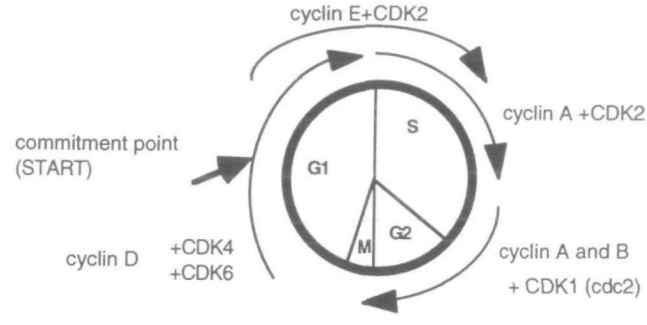

Figure 1. Combinatorial interactions of cyclins and cyclin dependent kinases (CDK) during the cell cycle.

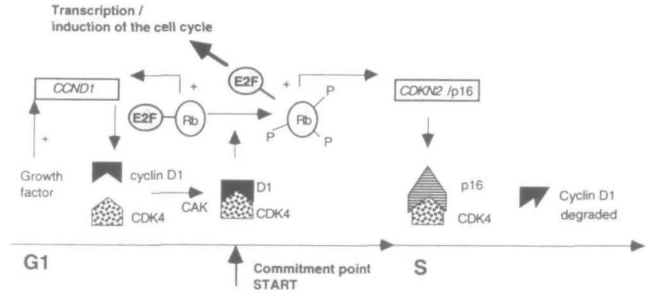

Figure 2. Model for G1 restriction-point control by cyclin D1: Growth factors induce the transcription of the cyclin D1 gene (*CCND1*) and positively regulate its assembly with CDK4 (and CDK6, not shown). The cyclin D1-CDK4 complex is activated by cyclin-activating kinase (CAK = cyclin H + CDK7) and the functional holoenzyme then phosphorylates the retinoblastoma protein, releasing and thus activating the E2F transcription factor. The *CDKN2* gene (encoding p16) inactivates CDK4 and induces accelerated turnover of unbound cyclin D1 during the S phase.

During the G1 phase, D-type cyclins appear to act as growth factor sensors, driving cells through the first restriction point (START in yeast), beyond which cells are committed to divide. Cyclin D1 together with specific kinases (CDK4, CDK6) and one particular kinase inhibitor (p16) appears to play a central role in the regulation of the proliferation status. Activated CDK4, through phosphorylation and complex formation with cyclin D1, inactivates the retinoblastoma protein (pRb). This results in the release from pRb of a transcription factor, E2F, which then activates many genes necessary for cell division. The p16 protein suppresses this process by competitively binding to the CDK4 molecule (Figure 2). Component genes of this specific control pathway are frequently compromised in malignant cells (for review see [1–5]).

The apparently ever-growing list of cell cycle regulators makes it increasingly difficult to keep abreast of this rapidly developing field, let alone for oncologists to think of clinical applications of these basic discoveries. However, some aspects are becoming clearer: with respect to cancer, one of the most important proteins is cyclin D1. It is now well established that cyclin D1 has oncogenic functions: constitutive overexpression in rodent cells can shorten the G1 phase [6], and similarly, in breast cancer cells arrested in G1, cyclin D1 induction is sufficient to complete the cell cycle and to shorten the G1-S phase [7]. Transfection of the cyclin D1 gene constructs into normal fibroblasts stimulates proliferation by reduction of the G1-S phase [6], and when transfected into normal fibroblasts with activated *H-ras*, it promotes the formation of fibrosarcomas in nude mice [8]. Moreover, mice carrying an active cyclin D1 transgene develop mammary hyperplasia and adenocarcinoma [9]. When overexpression of cyclin D1 is targeted to the lymphoid system, the transgenic mice develop lymphomas in the presence of *c-myc* overexpression [10, 11]. Very recently, cyclin D1 overexpression has been found at early stages of tumour development, in particular, in carcinoma *in situ* of the breast [12] and in bronchial epithelia of resection margins of non-small-cell lung cancer that were microscopically tumour-free [13].

The region of chromosome 11q13 encoding the cyclin D1 gene (*CCND1*) is frequently altered at the cytogenetic level in malignant disease. Increased gene copy numbers (gene amplification) have been found in breast, ovarian, bladder, esophageal, gallbladder, stomach, laryngeal, lung and liver cancers [1]. In esophageal [14], laryngeal [15] and head and neck tumours [16], *CCND1* amplification correlates with poor prognosis, a high incidence of distant metastasis and frequent recurrences. In almost all cases, amplification of the 11q13 region was associated with overexpression of cyclin D1. In lymphoproliferative disorders, another mechanism

may also lead to cyclin D1 overexpression: Translocation between the bcl-1 region, upstream of *CCND1*, and the immunoglobulin (Ig) heavy chain locus on chromosome 14 [17, 18] results in elevated transcription of *CCND1*, with high levels of cyclin D1 [19–22]. The translocation t(11;14)(q13;q32) occurs in 90% of mantle cell lymphomas and splenic lymphomas with villous lymphocytes as well as in a small percentage of certain aggressive lymphocytic and plasma cell leukaemias [19–21, 23–25].

This issue of *Annals of Oncology* carries an interesting paper expanding our knowledge of cyclin D1 in hairy cell leukaemia (HCL). De Boer et al. [26] report on cyclin D1 overexpression in the majority of patients with HCL using both molecular and genetic methods. These findings are in full agreement with the very recently published study by Bosch et al. [27]. Thirty-eight of 40 HCL patients in the two studies showed cyclin D1 overexpression. The level of expression is significantly lower than in mantle cell lymphomas; nevertheless, this finding is very interesting and rather unexpected, given the lack of evidence for molecular lesions involving *CCND1* in HCL.

Except for some lymphoproliferative disorders with the bcl-1 rearrangement, lymphoid tissues such as tonsils, spleen and lymph nodes apparently have no or very low levels of cyclin D1 expression [19, 28]. De Boer et al. [26] and Bosch et al. [27] were unable to explain the mechanism of cyclin D1 overexpression and *CCND1* deregulation in HCL. Neither structural anomalies within 11q13 (cytogenetic and molecular techniques) nor gene amplification were detected. Although rearrangements cannot be excluded outside the regions examined, translocation appears not to be a main mechanism responsible for cyclin D1 overexpression in HCL. Although the B-cell lineage of HCL is well established [29], there are several lines of evidence indicating some phenotypic and functional similarities between hairy cells and cells of monocyte/macrophage lineage [30], which normally express cyclin D1 [31]. Thus, it remains to be seen whether this cyclin D1 overexpression is at least partially responsible for the malignant behaviour of the hairy cells, or whether, as speculated by the authors, it might constitute a physiological characteristic shared by hairy cells. Additionally, it may represent an unspecific upregulation occurring as a result of the deregulated cycle network. Further work is therefore needed to establish the role of cyclin D1 in HCL.

For instance, the examination of allele specific *CCND1* expression in HCL would point to a possible role in malignancy. Were allelic imbalance observed in PCR-amplified cDNA it might be concluded that only one parental allele was responsible for cyclin D1 overexpression. This would lead to the assumption that there had been an alteration, such as a mutation within a *CCND1* control region or splice site (RNA processing). This phenomenon was recently demonstrated [32, 33]: Using immunostaining in a series of non-small-cell lung tumours, cyclin D1 was overexpressed in almost 30% of cases with no apparent structural abnormalities of *CCND1*, as assessed by Southern blotting and PCR. In these tumours we detected imbalances in allele-specific expression by RT-PCR and restriction fragment length polymorphism analysis of a *Hae*III polymorphism. Thus, genetic alteration of *CCND1* appears to be a key abnormality in lung carcinogenesis. Such a result in HCL would strengthen the evidence of a direct role for cyclin D1 in the disease.

Deregulation or loss of function of genes involved in the control of progression through the G1 restriction point (*CCND1, CDK4, CDKN2, RB1*) is common in human cancers. Conversely, lesions within the many other genes controlling aspects of the cell cycle (cyclin A, B, E, CDKs or CDK inhibitor genes) is much less frequent. In particular, overexpression of cyclin D1 has now been observed in around 50% of the most common and least treatable malignancies, including colorectal, head and neck, esophageal, breast, uterus and lung carcinomas, melanomas and sarcomas [14, 16, 32, 34–37]. In addition, various studies have demonstrated that blocking the action of cyclin D1 *in vitro*, with antibodies or antisense RNA prevents the growth of many tumour cell lines [6, 38]. However, evidence from mice in which both parental cyclin D1 genes have been knocked out suggests that complete loss of cyclin D1 function might have only limited effects on overall viability or normal cell proliferation in the adult [39]. These findings raise the exciting possibility that therapeutic agents directed against elements of this pathway, in particular cyclin D1, might prove not only successful in the treatment of common malignancies but also have relatively few side effects. Further research is needed to show whether such an approach might be transferred from the laboratory bench to patients. Should this turn out to be successful, then, without doubt, another editorial entitled 'Cyclin D1, the molecule of the century' will have to be written!

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